FINAL REPORT

Understanding How Nutritional Source and Behavioral State Interact to Influence Resistance to Abiotic Stressors in Honey Bees.

Christina Mogren
Postdoctoral Research Associate

James Ottea, Professor
Insecticide Toxicology Laboratory

Department of Entomology
Louisiana State University Agricultural Center
Baton Rouge, LA 70803

Aim 1: Does supplemental feeding increase heat, cold, and pesticide susceptibility?

Groups of 5- day old hive bees were fed 50% solutions of either honey, high fructose corn syrup (HFCS), HFCS supplemented with fresh pollen or sucrose syrup (SS). Then, after 5 days, they were exposed to hyperthermic or hypothermic conditions, and a commonly used pesticide in agriculture (imidacloprid). By calculating the mortality from each treatment, our goal was to determine if mortality from common abiotic stressors routinely encountered by honey bees increased from supplemental feeding practices. These data are relevant to beekeepers, especially in areas with harsh winters, hot summers, and/or heavy neonicotinoid use.

Results: Overall, mortality among groups of bees fed the various carbohydrate solutions was low (Figure 1). There was no difference in mortality between bees fed HFCS and HFCS and pollen. However, small but statistically significant, differences in mortality were measured between bees fed honey and the remaining treatments. This finding is interesting and will be explored further, perhaps in experiments with a duration >5 days. As such, it is likely that these differences would not be significant in a practical sense (i.e., to a commercial beekeeper).

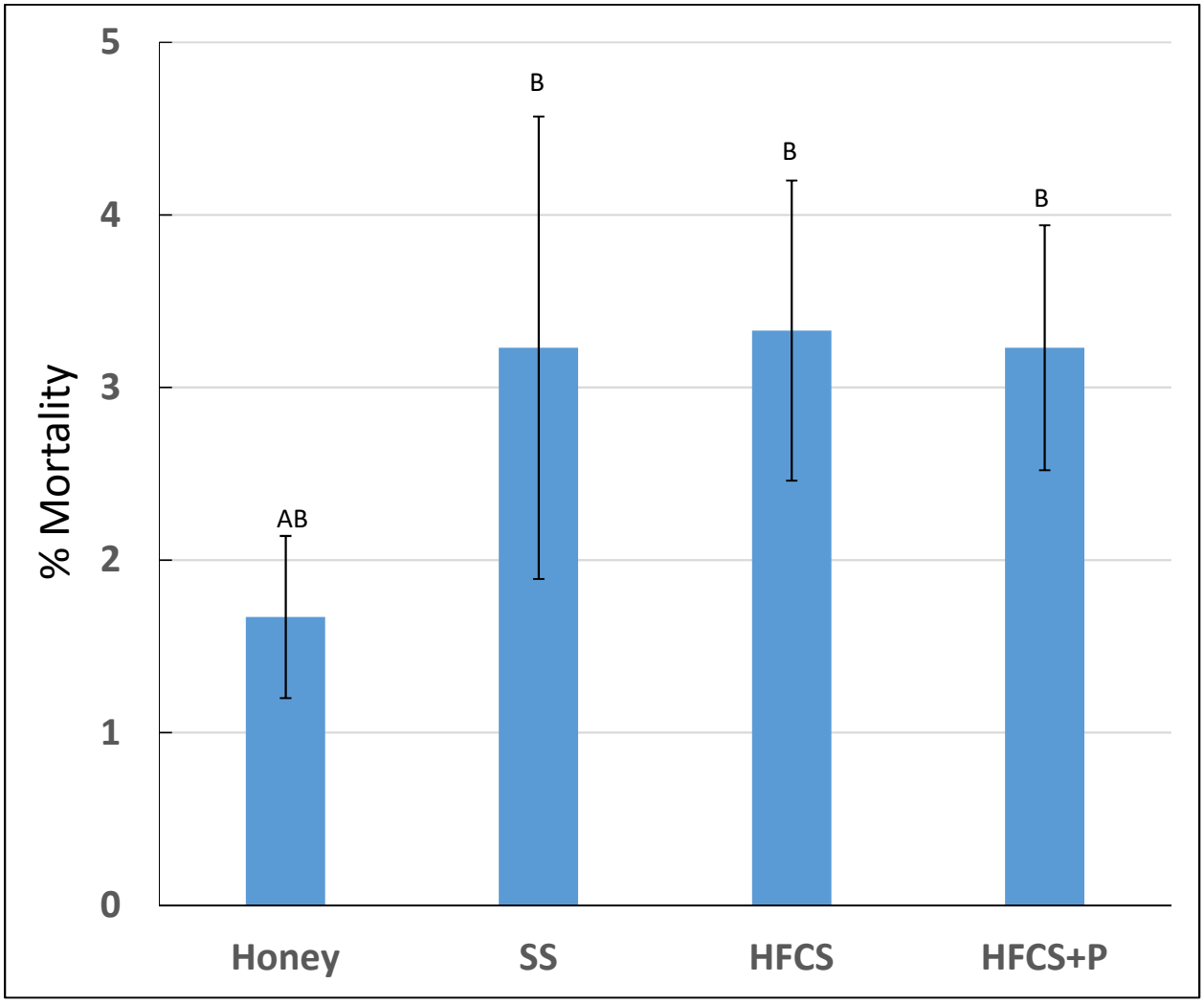

Figure 1: Effects of supplemental feeding on mortality measured after 5 days of exposure

In addition, supplemental feeding did not appear to affect the bees' response to temperature stress: feeding did not have a significant effect on mortality of bees reared 4 or 45°C (Figure 2).

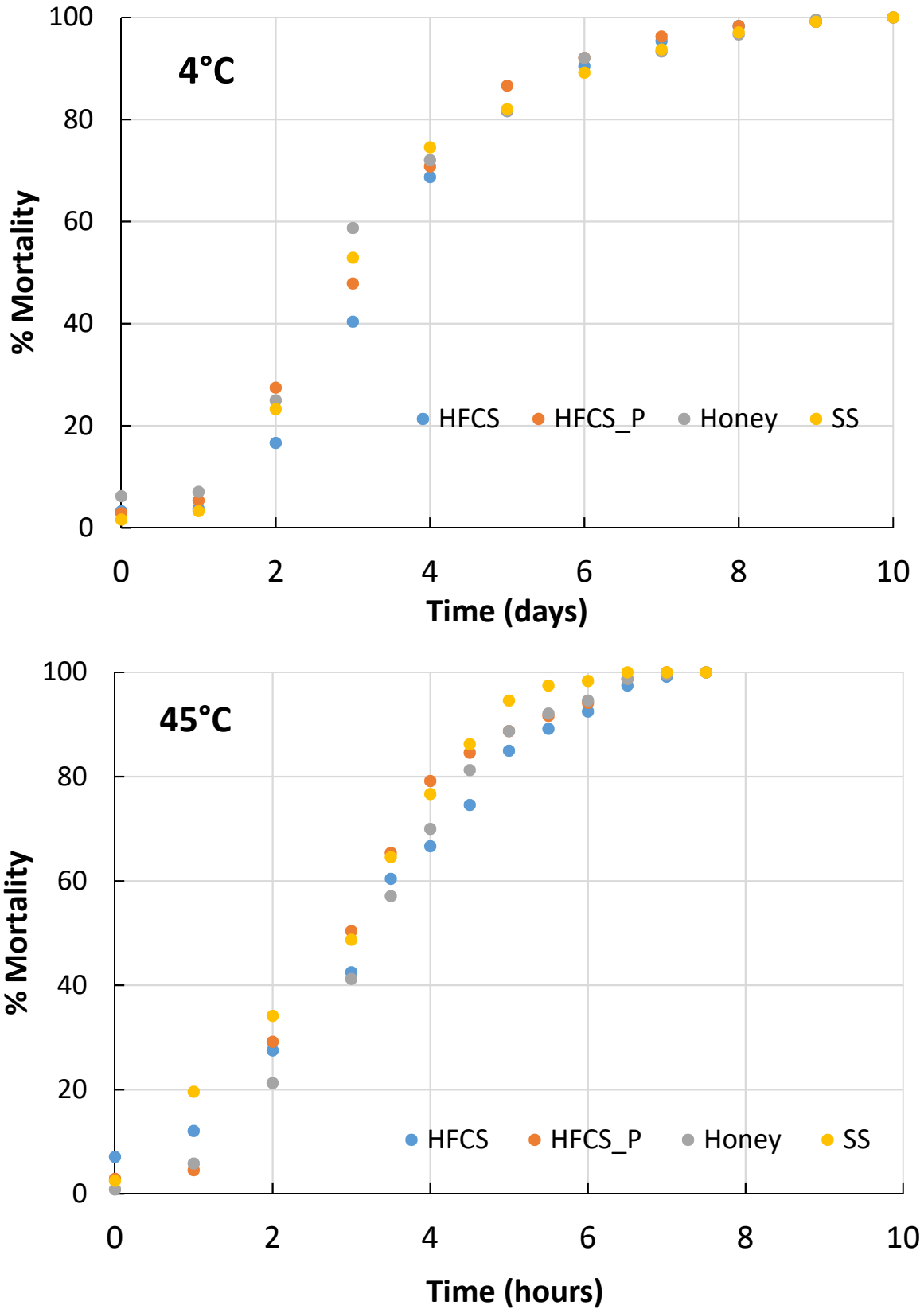

Figure 2: Effects of supplemental feeding on mortality following incubations at 4 and 45°C

However, our data indicate significant interactions between supplemental feeding and imidacloprid (IMCl) exposure (Figure 3). Mortality was significantly (5 times) greater in bees exposed to this insecticide after being fed sucrose (SS) 5 days after emergence. (Note that ion this analysis, a value of 1 indicates no differences between IMCl and control treatments.) This effect was especially apparent during the early ages (i.e., <10 days). The mechanism underlying this interaction is unknown, but might relate to differences in consumption of sugar sources in this experiment.

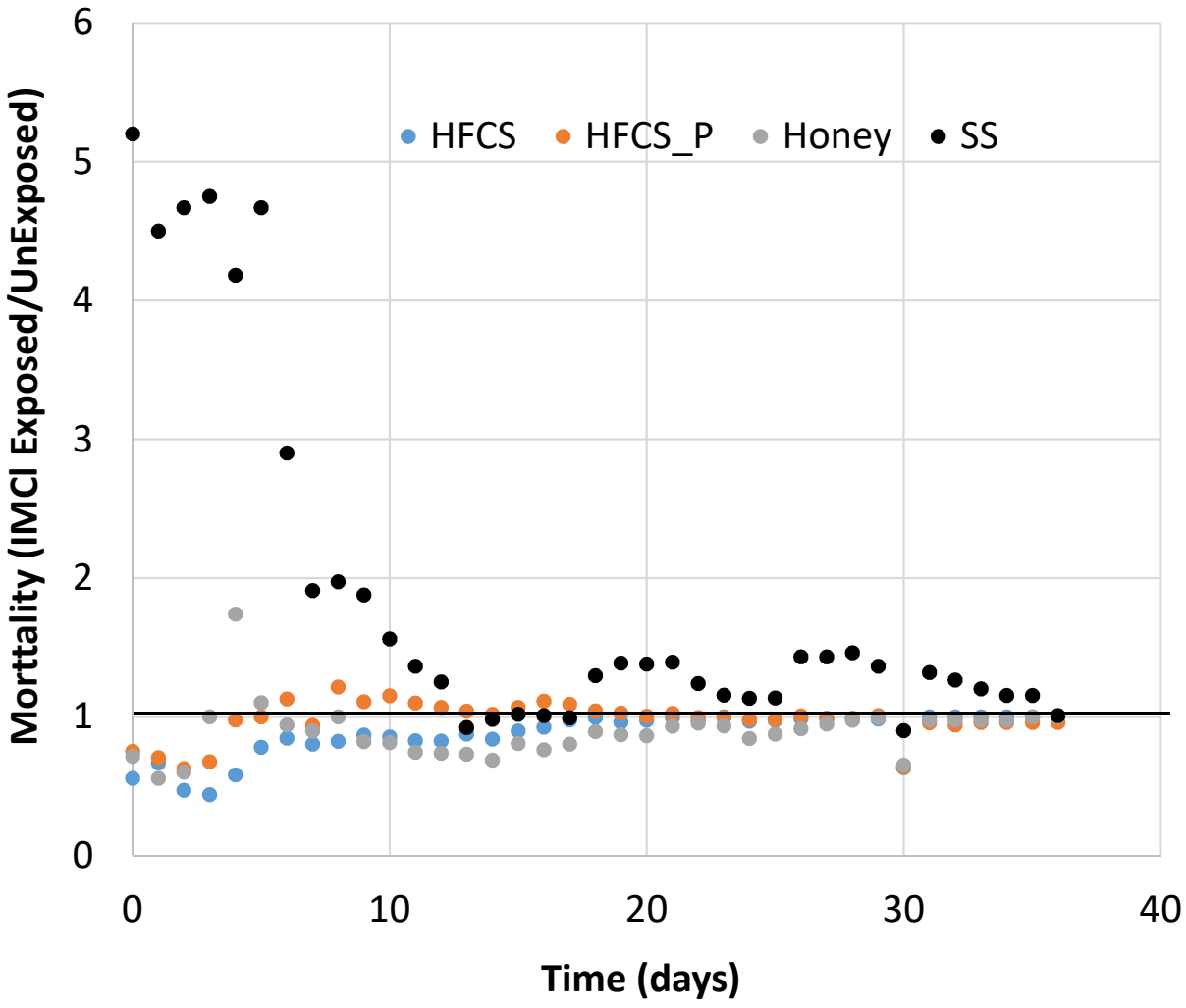

Figure 3: Effects of supplemental feeding on mortality following exposure to imidacloprid (IMCl)

Aim 2: Does supplemental feeding decrease detoxification/antioxidant enzyme activity and heat shock protein levels?

Groups of hive bees (10 bees collected from each of the 16 treatment combinations from each of six colonies (960 bees total) were stressed with heat, cold, or pesticide exposure (as above for Aim 1), then exposed for the median time to mortality. Abdominal homogenates from these bees were then analyzed for activities of esterases and superoxide dismutase, and levels of a 70 kilodalton heat shock protein (HSP70) was measured from heads.

Results: Supplemental feeding appeared to have little effect on esterase activities, which were similar among bees fed honey, SS HFCS, or HFCS+P (Figure 4). However, activities differed markedly between bees incubated at 4 and 45^{o}C. One interpretation of these data is that temperature, but not source of carbohydrates, is stressful for these bees, and this stress is reflected in decreased esterase activities.

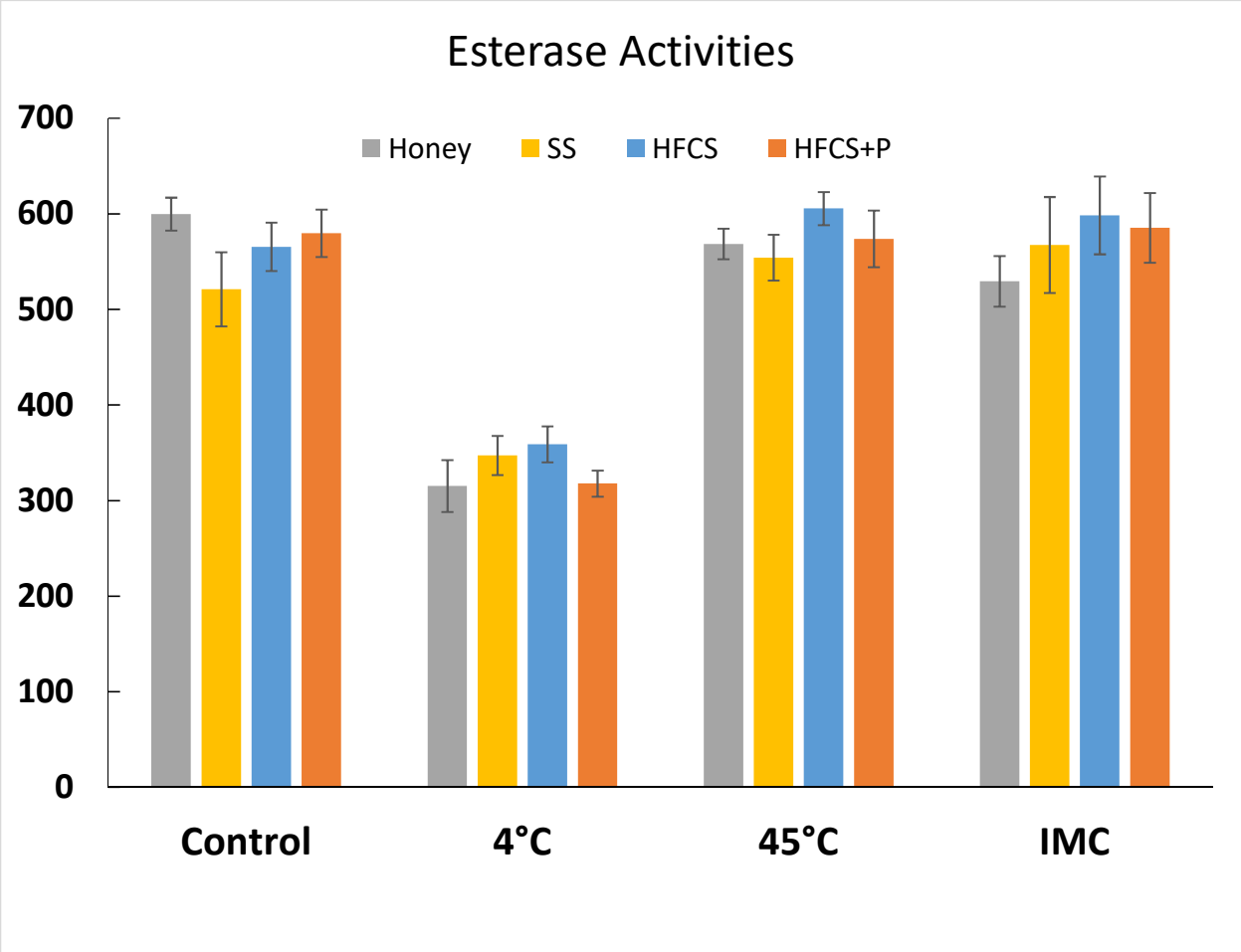

Figure 4: Effects of supplemental feeding, temperature, and imidacloprid exposure on esterase activities (nmoles naphthol formed/min·mg protein)

Similarly, supplemental feeding has little or no effect on activities of superoxide dismutase (Figure 5). In control bees, no significant differences were measured with respect to carbohydrate source. Similarly, no differences were measured among bees exposed to IMCl. However, superoxide dismutase activities were generally lower in bees fed HFCS than in controls. In addition, within treatments, activities differed depending on the supplemental feeding regimen. For example, activities were low in the thermally challenged bees that were fed HFCS (4ºC treatment) and SS or HFCS (4 and 45ºC treatments). Thus, it appears that temperature is a stressor but, unlike results with esterases, effects of temperature are exacerbated by certain supplemental feeding regimens.

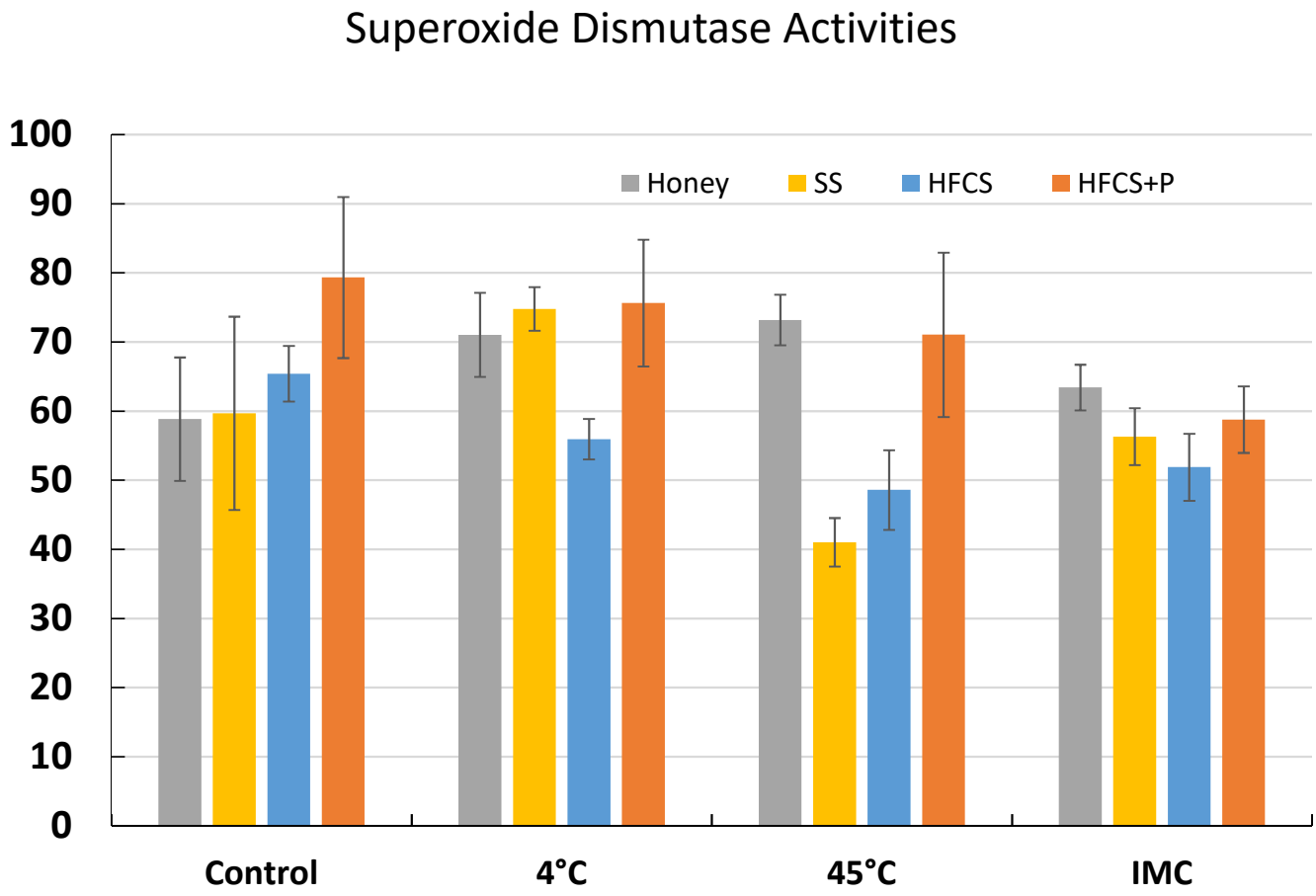

Figure 5: Effects of supplemental feeding, temperature, and imidacloprid exposure on superoxide dismutase activities (hydrogen peroxide formed/min·mg protein)

Finally, a third potential indicator of stress, heat shock protein (70 kD) was used to measure effects of dietary and thermal challenge in bees. Expression of this protein was variable both within and among treatments (Figure 6). In bees reared under normal conditions, expression varied depending on carbohydrate source and was highest in bees fed honey, and lowest in bees fed HFCS+ pollen. In addition, expression was variable in thermal treatments. In insects fed honey and sucrose, expression was lower than controls, but higher than controls in those fed HFCS and HFCS+ pollen. Finally, with the exception of bees fed honey, expression of HSP70 was higher in bees exposed to imidacloprid than those that were not exposed. This variability in response is interesting, but inexplicable at this point.

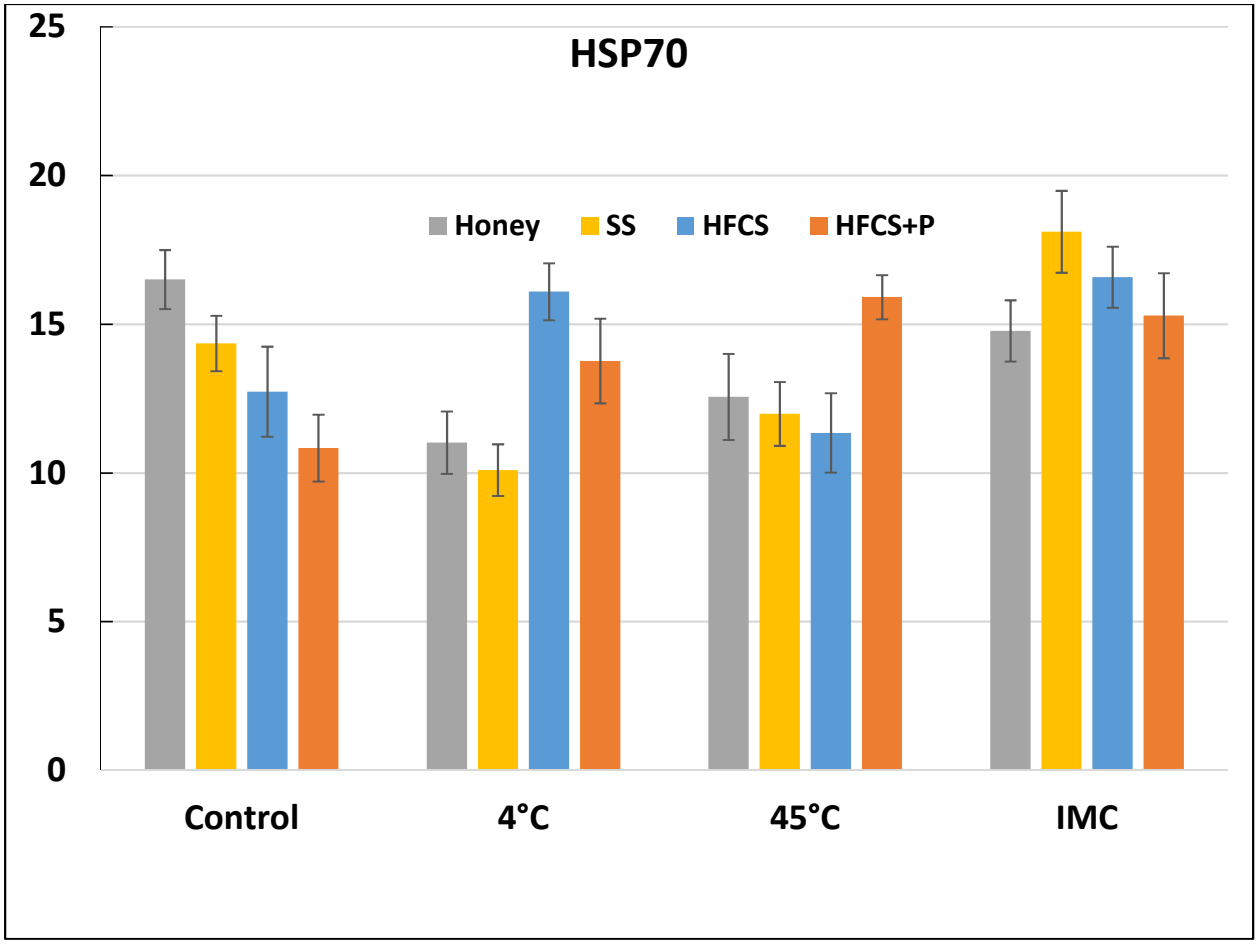

Figure 6: Effects of supplemental feeding, temperature, and imidacloprid exposure on expression of HSP70 (ng HSP70/2 µg protein)

Aim 3: Does early life feeding in hive bees decrease later life forager longevity?

We tested the hypothesis that exposing young hive bees to different carbohydrate sources would decrease longevity after transitioning to foraging tasks. Our prediction was that forager bees fed HFCS early in life would have decreased longevity relative to foragers fed SS, honey or HFCS plus pollen during early life. If feeding HFCS early in life decreases forager longevity, then precocious foraging may occur. The health of colonies with precocious foragers is markedly lower than colonies with a typical age structure suggesting atypical colony demography reduces productivity.

Measurement of forager mortality. Similar to aims 1 and 2, newly emerged bees were marked and placed back in their respective colony for 5 days and then subsequently fed HFCS, SS, or honey in the laboratory for 5 additional days. Bees from each treatment group received a unique color paint mark prior to colony reintroduction to identify which treatment they received. Next, these hive bees were returned to the hive and collected as foragers approximately 2 weeks later. These foragers were kept under normal laboratory conditions dead bees will be counted daily until mortality reaches 100%.

Results: Colony splits were completed that established 24 nuclear colonies at the field location (n=6 per feeding treatment). Bees fed sucrose (SS) early in life were at a significant survival

disadvantage compared to other treatments, until approximately two weeks after feeding when mortality was similar among treatments (Figure 7).

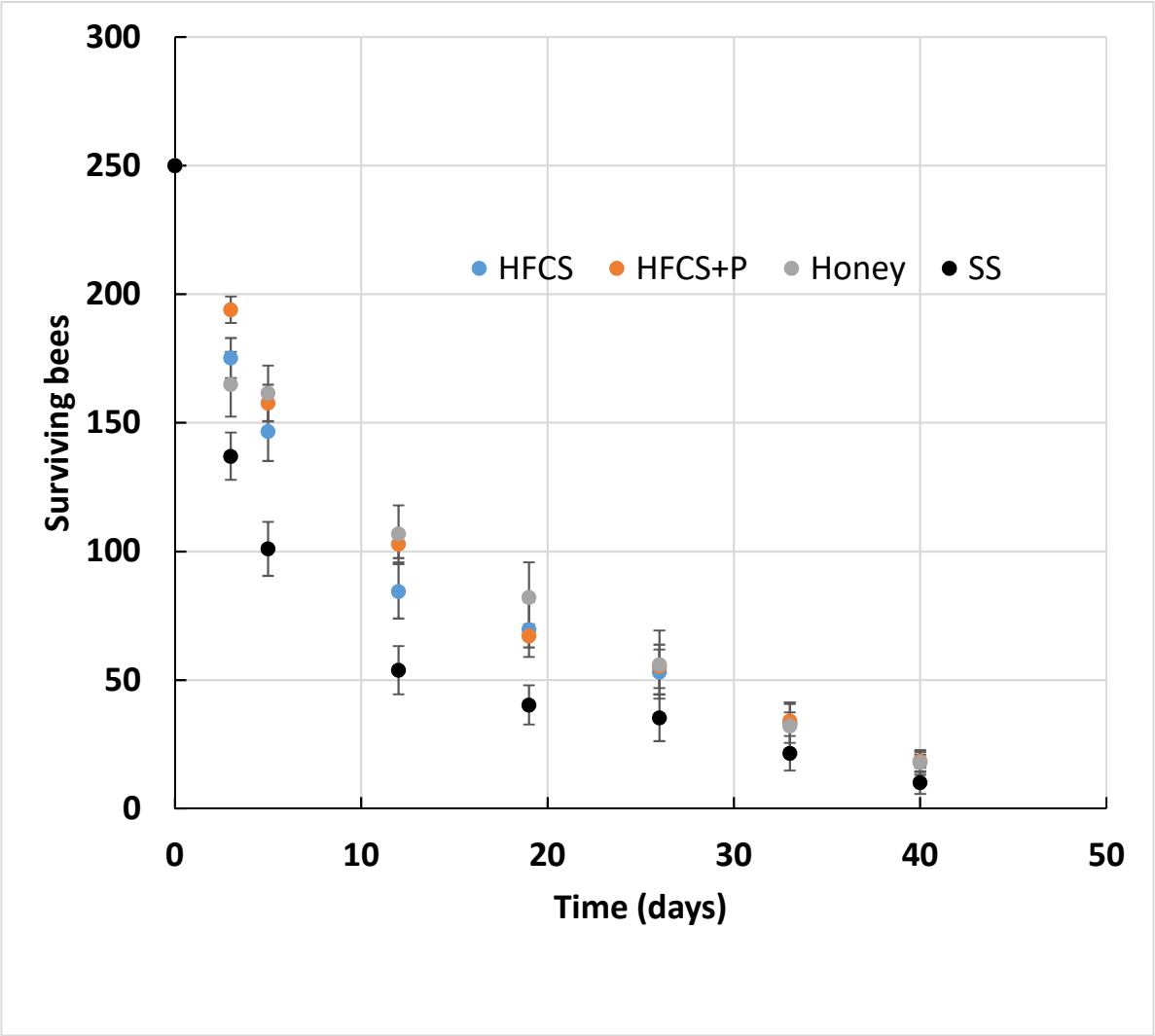

Figure 7: Effects of supplemental feeding on mortality in foraging bees.

Conclusions: There were two main objectives of this research. The first was to measure effects of feeding bees various carbohydrate sources early in life. As effects, we measured mortality (Figure 1), and foraging longevity/success (Figure 7) after five days exposure to honey, a seemingly innocuous food source, and sucrose and high fructose corn syrup, two carbohydrate sources of questionable quality. Survival in young, 10 day old bees that were fed these solutions was slightly higher in honey treatments relative to the others. However, mortality among all treatments was very low (less than 4%). At most time points, bees fed honey had the greatest survival as foragers, but differences were only significant relative to the sucrose- fed bees, which had dramatically lowered survival, especially at early time points. One interpretation of these findings is that supplemental feeding with sucrose (and to a lesser extent, HFCS) is detrimental, but differences are manifest as shortened lifespans among foragers. This is perhaps the major finding of this research, and will be followed up.

The second major objective was to screen expression of three proteins as markers for stress in bees. The first group of proteins, esterases, are traditional detoxifying enzymes, but are also associated with metabolism of endogenous substrates (e.g., acetylcholine) in bees. Previous reports suggest that activities of these enzymes are increased in bees following exposure to insecticides, but to our knowledge, these enzymes have not been examined as markers for stress. In our studies, no effects of supplemental feeding on these enzymes were apparent: activities were remarkably similar within treatment groups. In addition, bees exposed to insecticide (imidacloprid) expressed activities similar to the control. However, there was a significant decrease in activities when bees were exposed to 4^{o} conditions. This finding is somewhat surprising and suggests that activities decline in response to low temperature stress.

Activities of a second enzyme, superoxide dismutase, were measured as an indicator of oxidative stress. Activities were variable among treatments but did not differ significantly between control and imidacloprid- exposed bees. However, the greatest differences within treatments were measured in thermally challenged bees, for which activities were lowest for HFCS (4^{o}C treatment) and SS or HFCS (4 and 45^{o}C treatments).

Finally, expression of heat shock protein (HSP70) was measured in our bees. These proteins are ubiquitous, and are typically upregulated in cells following many forms of stress. In our experiments, expression was variable with respect to both feeding and abiotic stressors. In control bees, levels were highest in honey- fed and lowest in HFCS+pollen- fed bees, which was unexpected. Levels were generally higher in bees exposed to imidacloprid, especially those fed SS, HFCS, and HFCS with pollen. However, levels were lowest in temperature challenged bees that were fed honey and sucrose solutions. Clearly, interactions among stressors and expression of all three proteins is complex, and discerning patterns from these results appears difficult at this time.

Acknowledgment- We greatly appreciate your support (and patience!) during the past two years.